A pharmacological profile of the aldehyde receptor repertoire in rat olfactory epithelium

Ricardo C. Araneda, Zita Peterlin, Xinmin Zhang, Alex Chesler and Stuart Firestein

Department of Biological Sciences, Columbia University, New York, NY 10027, USA

Several lines of evidence suggest that odorants are recognized through a combinatorial process in the olfactory system; a single odorant is recognized by multiple receptors and multiple odorants are recognized by the same receptor. However few details of how this might actually function for any particular odour set or receptor family are available. Approaching the problem from the ligands rather than the receptors, we used the response to a common odorant, octanal, as the basis for defining multiple receptor profiles. Octanal and other aldehydes induce large EOG responses in the rodent olfactory epithelium, suggesting that these compounds activate a large number of odour receptors (ORs). Here, we have determined and compared the pharmacological profile of different octanal receptors using Ca^{2+} imaging in isolated olfactory sensory neurones (OSNs). It is believed that each OSN expresses only one receptor, thus the response profile of each cell corresponds to the pharmacological profile of one particular receptor. We stimulated the cells with a panel of nine odorants, which included octanal, octanoic acid, octanol and cinnamaldehyde among others (all at 30 μm). Cluster analysis revealed several distinct pharmacological profiles for cells that were all sensitive to octanal. Some receptors had a broad molecular range, while others were activated only by octanal. Comparison of the profiles with that of the one identified octanal receptor, OR-I7, indicated several differences. While OR-I7 is activated by low concentrations of octanal and blocked by citral, other receptors were less sensitive to octanal and not blocked by citral. A lower estimate for the maximal number of octanal receptors is between 33 and 55. This large number of receptors for octanal suggests that, although the peripheral olfactory system is endowed with high sensitivity, discrimination among different compounds probably requires further central processing.

(Received 12 November 2003; accepted after revision 9 January 2004; first published online 14 January 2004)
Corresponding author R. C. Araneda: Department of Biological Sciences, Columbia University, New York, NY 10027, USA. Email: rca23@columbia.edu

Terrestrial vertebrates occupy an odour-rich environment of considerable variety and complexity. Olfactory systems have evolved to meet the numerical and physical challenges of chemical detection and discrimination. They operate over a dynamic range of several orders of stimulus magnitude and can recognize an enormous array of low to medium molecular weight organic compounds. Theoretically, there is no limit to the number and variety of compounds that can be considered odorants. If a receptor expressed in a primary olfactory sensory neurone (OSN) binds a compound, that compound will be, by definition, an odour. The large number of receptors used in olfactory systems and the combinatorial strategy of overlapping affinities assures that the number of possible receptor combinations is larger, by several orders of magnitude, than the number of known chemicals. In some ways this is analogous to vision in which it is possible to see thousands, perhaps millions, of hues with only three receptors of overlapping bandwidth. However, it also differs from vision, hearing and other sensory systems in that the stimulus does not vary along a single physical dimension (i.e. wavelength, frequency). Rather, the stimulus varies along multiple dimensions including, but not limited to, molecular shape and size, functional group, charge, hydrophobicity and atomic composition.

Olfaction utilizes a large family of G-protein-coupled receptors (GPCRs) expressed in specialized primary sensory neurones residing in a thin neuro-epithelium in the nose (Buck, 1992). The odour receptor (OR) family of genes, comprising some 1100 functional genes in the mouse, is the largest family of GPCRs in the mammalian genome (Mombaerts, 1999; Zhang & Firestein, 2002). Nonetheless, this is nowhere near the number of compounds possessing an odour quality. Thus,

DOI: 10.1113/jphysiol.2003.058040

it is generally accepted that a combinatorial strategy must be employed, in which most odorants are recognized by several receptors and most receptors recognize multiple related compounds (Malnic *et al.* 1999; but see Hamana *et al.* 2003). A particular odour percept is then produced in higher brain centres that 'decode' the combination of activated receptors.

It is clear from this model that the primary event of receptor–odorant interaction is critical in determining the range of odorant sensitivities. Several lines of evidence suggest that, with a few exceptions (Rawson *et al.* 2000), each OSN expresses only one OR gene (Serizawa *et al.* 2003). All cells expressing the same receptor converge onto one or a few restricted targets, known as glomeruli, in the olfactory bulb (Mombaerts, 1999). This suggests that the molecular range, or pharmacological profile, of each OR defines the receptive field of each glomerular unit. As with other sensory systems, defining the receptive field of olfactory neurones would provide critical information about the manner in which the stimulus is encoded.

Progress in this area has been slowed by the lack of a reliable high-throughput OR expression system in which a pharmacological programme of ligand screening could be carried out on many receptors. Thus, there are only a handful of identified mammalian receptors for which a few cognate ligands have been determined and confirmed (Krautwurst *et al.* 1998; Zhao *et al.* 1998; Murrell & Hunter, 1999; Wetzel *et al.* 1999; Kajiya *et al.* 2001; Touhara, 2001; Bozza *et al.* 2002; Gaillard *et al.* 2002). Previously, using an adenovirus expression system, we were able to drive the expression of OR-I7 in a large number of OSNs (Zhao *et al.* 1998). This enabled us to make a comprehensive investigation of the molecular receptive range of this receptor and to identify critical chemical attributes of the ligands that predict the likelihood of receptor-ligand activity. This exercise has allowed us to define an OR-I7 profile based on function rather than gene sequence (Araneda *et al.* 2000). We found that OR-I7 displays a high specificity for certain molecular features (e.g. only aldehydes were active), and a high tolerance for others (e.g. unsaturated 8-carbon aldehydes were as effective as the saturated octanal in activating the receptor).

Here we take a different approach to the same problem. Using a set of compounds that were chosen for their chemical disparity, we asked if it were possible to identify distinct chemical profiles of receptors by screening a large number of cells with the same compound set. Even though we are ignorant of the genetic identity of the OR protein expressed, we can rely on the principle that only one OR gene is being expressed by any given neurone, and the response of that neurone is a faithful reflection of the receptive range of the receptor it is expressing. With this strategy we have been able to distinguish at least 33 distinct types of receptors, all of which bind octanal, but each of which differs in its sensitivity to other test compounds.

Methods

Isolation of sensory neurones

All animal procedures conformed to Columbia University guidelines for care and use of animals. Sensory neurones were isolated from 6- to 8-week-old Sprague-Dawley rats of both sexes. Two experimental groups of animals were used to compare the pharmacological profile of OR-I7 to that of other octanal receptors. These included animals that were infected with adenovirus containing the OR-I7, following the same protocol previously used (Zhao *et al.* 1998; Araneda *et al.* 2000), as well as uninfected animals.

Animals were overdosed with anaesthetics (ketamine, 90 mg kg^{-1}; xylazine, 10 mg kg^{-1}, I.P.) and decapitated. The head was cut open sagitally and the septum was removed to expose the medial surface of the olfactory turbinates. The epithelium was dissected out and placed in an oxygenated divalent-free rat Ringer solution (mM: 145 NaCl, 5.6 KCl, 10 Hepes, 10 glucose, 4 EGTA, pH 7.4). For infected animals, the epithelium was dissected under a fluorescent microscope (see below). The tissue was then incubated at 37°C for 45 min in 5 ml of divalent-free Ringer solution containing 5 mg ml^{-1} bovine serum albumin (Sigma-Aldrich, St Louis, MO, USA), 0.5 mg ml^{-1} collagenase, 22 U ml^{-1} dispase (Gibco BRL, Grand Island, NY, USA) and 50 μg ml^{-1} deoxyribonuclease II (Sigma). The tissue was then transferred to a normal rat Ringer solution (mM: 138 NaCl, 5 KCl, 1 $CaCl_2$, 1.5 $MgCl_2$, 10 Hepes, 10 D-glucose, pH 7.4) and the cells were dissociated by tapping the tube containing the tissue. Cells (in 400 μl volume) were plated onto concanavalin A (Sigma-Aldrich, 10 mg ml^{-1})-coated glass coverslips and placed in 35 mm Petri dishes. After allowing the cells to settle for 20 min, 2 ml of culture medium was added to each dish and the dishes were placed at 37°C in a CO_2 incubator for at least 1 h. The culture medium consisted of DMEM/F12 (Gibco BRL) supplemented with 10% fetal bovine serum, 100 μM ascorbic acid, 1X insulin–transferrin–selenium (Gibco BRL), 2 mM glutamine, 100 U ml^{-1} penicillin and 100 μg ml^{-1} streptomycin (Gibco BRL).

Ca^{2+} imaging recordings

Calcium imaging recordings were performed as described elsewhere (Yuste *et al.* 2000). After incubation, the culture

medium was removed and the cells were loaded with Fura-2 AM (5 μM) plus pluronic acid F127 (80 μg ml^{-1}; Molecular Probes, Eugene, OR, USA) in rat Ringer solution. Cells were loaded in the dark for 45 min, at room temperature. After washing with fresh Ringer solution, the coverslip was mounted onto a recording chamber. Imaging was carried out at room temperature on an inverted fluorescence microscope (IMT-2 Olympus, Tokyo, Japan) equipped with a SIT camera (C2400-08, Hamamatsu Photonics, Hamamatsu, Japan) connected to a frame grabber (LG-3, Scion, Frederick, MD, USA) on a Macintosh computer. The NIH Image software was used for data acquisition and analysis (NIH, Bethesda, MD, USA). Customized macros were written for shutter control (Uniblitz, Vincent Associates, Rochester, NY, USA) and time-lapse imaging. Recordings were made at 380 nm excitation and 510 nm emission. Images were taken every 4 s and 3 frames were averaged. The recording chamber was continuously perfused with oxygenated Ringer solution by means of a peristaltic pump. Odorant solutions were freshly prepared in rat Ringer solution by dilutions from odorant stocks made in DMSO and applied through syringes connected to the perfusion system via a manifold. Odours were applied for 8 s in enough volume to completely replace the solution in the chamber (200 μl). The odorant concentration, unless otherwise noted, was 30 μM and stimuli were applied at intervals of at least 1 min. Data is shown as the fractional change in fluorescent light intensity: $\Delta F/F_0$ or $(F - F_0)/F_0$, where F is the fluorescent light intensity at each point and F_0 is the value of emitted fluorescent light before the stimulus application (baseline). Odorants were applied randomly; however, in most of the figures the order of odour presentation was modified for illustrative purposes. All odorants were purchased from Sigma-Aldrich, except 2,5,7-trimethyl-2-octenal, neral and geranial.

Data analysis

Isolated OSNs were stimulated with a panel of odorants that included saturated, unsaturated, and branched aldehydes and some non-aldehyde odorants (Fig. 1) while responses were monitored with Ca^{2+} imaging. Some of these molecules were previously used to define the molecular range of one of the receptors for octanal, OR-I7, using EOG recordings (Araneda *et al.* 2000). To compare the profile of OR-I7 with that of other putative receptors for octanal we infected OSNs with a bicistronic adenoviral construct encoding both OR-I7 and green fluorescent protein (GFP) (Zhao *et al.* 1998; Araneda *et al.* 2000). Thus, cells expressing OR-I7 could be identified by expression of the GFP marker (GFP+ cells). Although infected OSNs also express their endogenous receptor, these will be a random selection from among the approximately 1200 rat ORs (S. Firestein, unpublished observations). For example, although citral is not an OR-I7 agonist, 37% of the GFP+ cells responded to citral (Table 1). However, the size of the response to citral in GFP+ cells did not correlate with the size of the response to octanal (data not shown) and a similar fraction of the GFP– cells (34%) also responded to citral (Table 1). Moreover, even upon increasing the concentration of citral to 500 μM, in the same group of cells the percentage of GFP+ and GFP– cells responding to citral was comparable (data not shown). Taken together these results indicate that the citral response observed in a subset of GFP+ cells is likely to be due to the activation of endogenous receptors sensitive to citral (and other compounds as well), which are prevalent among OSNs, and hence were present in some of the randomly infected OSNs (see also Figs 4 and 5).

Responses were quantified using macros included in the Igor Pro software (WaveMetrics, Lake Oswego, OR, USA). An odorant was considered to elicit a response only if the change in $\Delta F/F_0$ was higher than two times the value of the standard deviation of the baseline and the decrease in $\Delta F/F_0$ lasted more than 20 s. For most of the experiments the amplitude of the responses was measured as the difference between the values of $\Delta F/F_0$

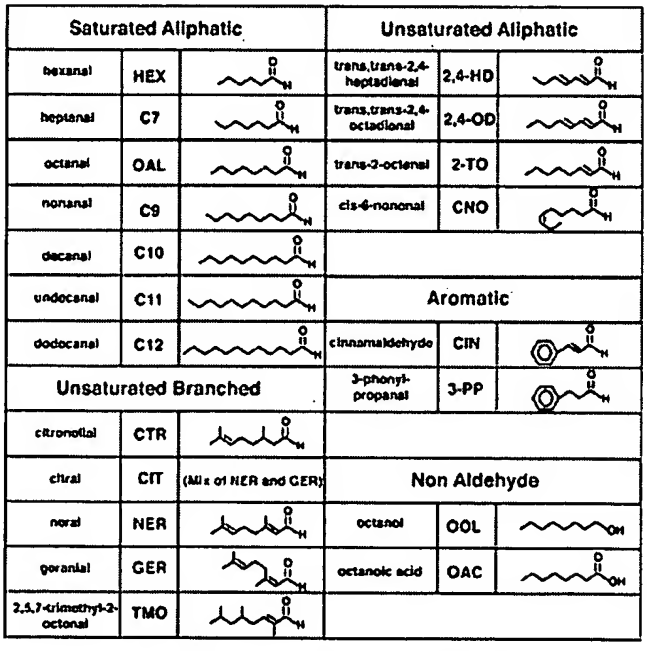

Figure 1. Panel of odorants used to characterize the pharmacological profiles of receptors for octanal
The abbreviation, given for each compound, is that used in the subsequent figures.

Table 1. GFP+ (OR-I7) and GFP– cells tested with different aldehydes and non-aldehyde compounds in OR-I7-infected animals

		No response	<50%	50–100%	>100%	Total	% Responding
GFP positive	HEX	3	0	0	0	3	0
	C7	3	8	12	0	23	87
	OAL	—	—	—	—	73	—
	C9	8	11	10	2	31	82
	C10	20	6	1	1	28	29
	C11	21	2	1	0	24	13
	C12	5	0	0	0	5	0
	CIT	22	11	2	0	35	37
	CTR	3	4	25	5	37	92
	2,4-HD	0	3	3	5	11	100
	2-TO	1	0	17	7	25	96
	2,4-OD	1	0	7	25	33	97
	TMO	11	0	0	0	11	0
	CNO	0	2	1	1	4	100
	OAC	7	0	0	0	7	0
	OOL	6	0	0	0	5	0
	CIN	5	0	0	0	5	0
GFP negative (OAL responding)	HEX	5	0	0	0	5	0
	C7	20	18	15	7	60	67
	OAL	—	—	—	—	106	-
	C9	10	12	27	9	58	83
	C10	23	15	9	10	57	60
	C11	31	9	4	5	49	37
	C12	3	0	0	0	3	0
	CIT	58	12	10	8	88	34
	CTR	37	18	22	4	81	54
	2,4-HD	12	12	7	4	35	66
	2-TO	16	9	25	18	66	76
	2,4-OD	24	9	15	23	71	66
	TMO	23	3	0	2	28	18
	CNO	0	0	2	1	3	100
	OAC	9	2	3	6	20	55
	OOL	13	1	4	4	22	41
	CIN	2	1	0	1	4	50

All cells were tested with at least 3 compounds, including octanal. The size of the responses for the different compounds is expressed relative to the response to octanal (100%). A larger percentage of GFP+ cells, compared to GFP– cells, responded to the unsaturated aldehydes 2-TO, 2,4-HD and 2,4-OD and the unsaturated-branched aldehydes CNO and CTR.

of the peak of the response and the baseline. For the data shown in Figs 2*B* and 4*B*, the amplitude of the response was obtained by integrating the area under the curve, using a window of 90 s starting at the peak of the response. Statistical significance was assessed using the Student's unpaired *t* test. For the dose–response relations the data were fitted to the Hill equation. Data are shown as means ± S.E.M. of at least three different cells. In preliminary experiments we found that on average 20% of the cells in the recording field were viable OSNs, as measured by the response to both a high-KCl stimulus (50 mM) and the phosphodiesterase inhibitor 3-iso-butyl-1-xanthine (IBMX, 2 mM). In most of the experiments odorant responses were compared to the responses to octanal, and repeated applications of octanal allowed us to check for the possible rundown of the responses. Those cells in which the response to octanal decreased more than 60% were discarded.

For cluster analysis the data were analysed using the EPICLUST software by directly loading spreadsheets made with the Excel software into the http://ep.ebi.ac.uk/EP/server. For each cell a response was assigned a value of 1 and no response a value of 0. Thus, all the responses, regardless of their size compared with that of octanal, had the same value. The same data transformation was applied for the cells shown in Fig. 3.

Results

The odorant octanal and other aldehydes produce activation of a large number of glomeruli in the olfactory bulb (Johnson *et al.* 2002; Xu *et al.* 2003), suggesting

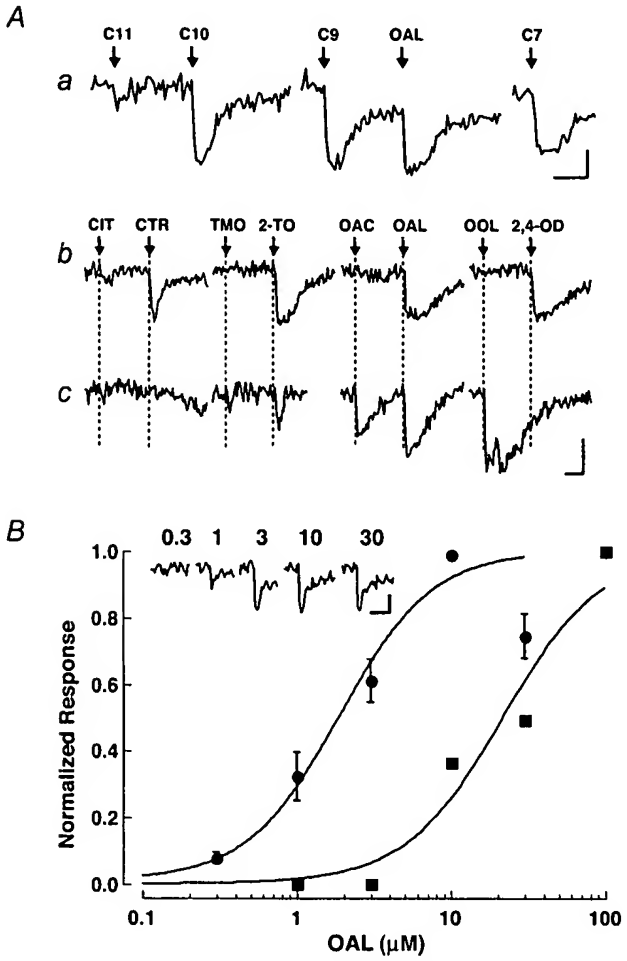

Figure 2. Pharmacology of OR-I7-expressing cells and cells expressing other receptors for aldehydes

A, comparison of the responses to octanal and other odorants in cells expressing OR-I7. For this and the following figures odorants were applied for 8 s, beginning at the time indicated by the arrow. Cells were activated by C7–C11 aldehydes (*a*) and the unsaturated aldehydes, CTR, 2-TO and 2,4-OD, but not by CIT, TMO, OAC and OOL (*b*). *Ac*, activity profile for a GFP– cell. This cell responded to octanal and 2-TO, but it also responded to OAC and OOL, while it did not respond to CTR and 2,4-OD. All compounds were tested at 30 μM, except OAC and OOL (at 1 mM). The calibration bar is 6% Δ*F*/*F* (vertical) and 1 min (horizontal) and is the same for *b* and *c*. *B*, dose–response-relation for OAL in cells expressing OR-I7 (●) and a GFP– cell (■). Responses were normalized to the largest response (10 and 100 μM, respectively) and fitted to the Hill equation. Responses in OR-I7 are shifted to the left, indicating greater sensitivity for octanal. Inset, dose-dependent increases in calcium signal induced by OAL in a cell expressing OR-I7. The calibration bar is 4% Δ*F*/*F* (vertical) and 1 min (horizontal).

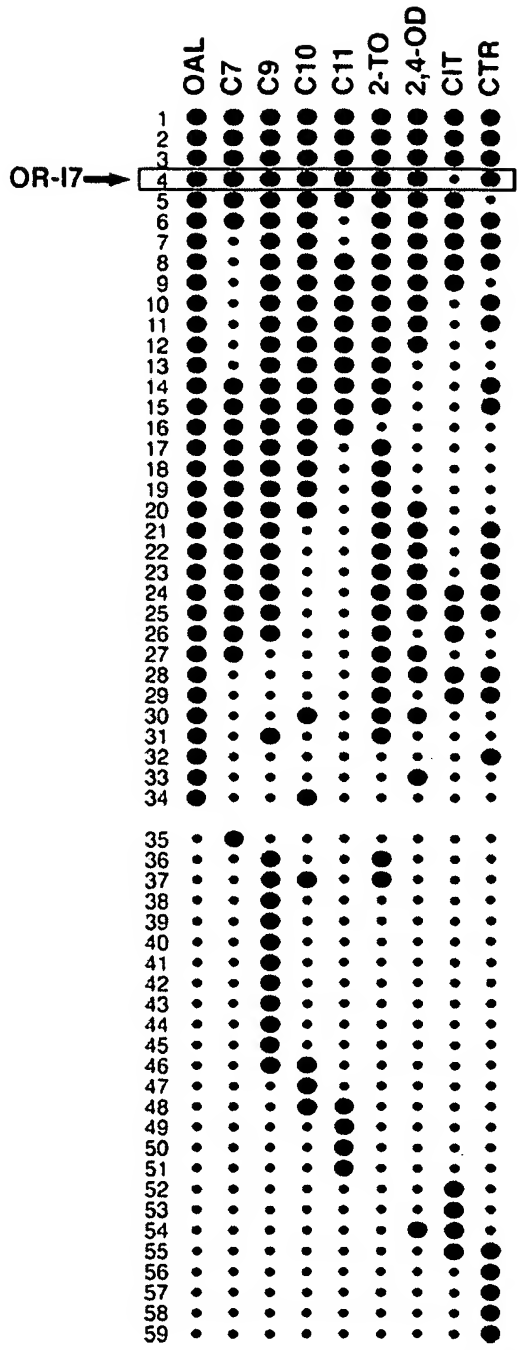

Figure 3. Aldehyde receptors can discriminate closely related compounds

Pharmacological profiles of 59 GFP– cells, tested with a panel of 9 aldehydes. Responses are indicated by a red circle and no response, by a black, smaller circle. In this group only cell 4, outlined by a rectangle, exhibited the pharmacological profile of OR-I7; it was activated by all aldehydes tested except CIT. Other cells exhibited varying degrees of sensitivity. The lower section of the panel shows a group of 25 cells that did not respond to OAL, but responded to other aldehydes in the panel. These cells were all rather narrowly tuned. All compounds were tested at 30 μM.

that these compounds activate a large number of ORs. We have used this wide-ranging response to aldehydes, in particular octanal, as the basis for identifying and defining multiple receptor profiles in isolated OSNs. In a first set of experiments we identified different receptors by their sensitivity to closely related aliphatic aldehydes. Many of these compounds were previously used to define the molecular range of an identified receptor for aldehydes, OR-I7 (Araneda *et al.* 2000). In addition, we further extended the distinction between different aldehyde receptors by using a panel that includes distinct molecules.

A receptor for aldehydes can discriminate among closely related molecules

Several studies have proposed that ORs can recognize odorants with very different structures (Sicard & Holley, 1984; Duchamp-Viret *et al.* 1999; Ma & Shepherd, 2000). However, a thorough characterization of the receptive field of one receptor, OR-I7, shows clear discrimination among aliphatic aldehydes with only subtle modifications in the vicinity of the carbonyl group (Araneda *et al.* 2000). Still other studies have shown varying degrees of selectivity in mammalian OSNs (Sato *et al.* 1994; Bozza & Kauer, 1998; Kaluza & Breer, 2000). In a first set of experiments we determined the receptive field of OR-I7 using Ca^{2+} imaging and found that the profiles of responses were in accord with our previous EOG studies (Araneda *et al.* 2000). Thus, cells expressing OR-I7 responded to octanal and other aldehydes with 7- to 11-carbon backbones (30 μM, Fig. 2*Aa*), but did not respond to the shorter (<7) or longer (>11) aldehydes (Table 1). Similarly, cells expressing OR-I7 also responded to the unsaturated 8-carbon aldehydes, *trans*-2-octenal and 2,4-octadienal, and the unsaturated and branched, citronellal (Fig. 2*Ab*). None of the cells expressing OR-I7 responded to 8-carbon molecules bearing different functional groups such as octanoic acid or octanol (both at 1 mM, Fig. 2*Ab*). Also, none of the cells expressing OR-I7 responded to the aldehydes, 2,5,7-trimethyl-*trans*-2-octenal or cinnamaldehyde, which has been recently described as an agonist for this receptor in mouse (Bozza *et al.* 2002). This discrepancy may be due to differences in the level of receptor expression achieved with the adenovirus and in the gene targeting experiments. We did find a large number of cells that responded to octanal but which otherwise had pharmacological profiles that were distinct from OR-I7. Some of those cells were less discriminatory and they will be discussed below; for illustration one such aldehyde-responsive cell is shown in Fig. 2*Ac*.

To test the possibility that other receptors for aldehydes could also discriminate among closely related compounds we challenged OSNs with a set of nine aliphatic aldehydes, including octanal. The stimulating panel included saturated aldehydes of increasing carbon chain length (C7–C11), 8-carbon unsaturated (*trans*-2-octenal and 2,4-octadienal), and unsaturated-branched (citral and citronellal) aldehydes. The response profiles of a group of 59 selected cells are shown in Fig. 3. In this group, cell 4 was the only one to exhibit the expected profile of OR-I7 (Fig. 3, arrow). For comparison, we included in this group 25 cells that did not respond to octanal but did respond to another component of the panel. In general these cells were rather narrowly tuned to one or a few odorants; 20/25 cells responded to only a single odorant in the panel.

Inspection of the profiles of the responding cells revealed some surprising selectivity among these receptors. The unsaturated aldehydes *trans*-2-octenal and 2,4-octadienal, and the unsaturated-branched aldehydes citral and citronellal, differ only by the presence of one extra double bond, yet several cells were able to distinguish between these aldehydes despite being activated by all or most of the saturated aldehydes in the series C7–C11. Moreover, a set of these cells (28, 29, 32, 33) was activated strictly by 8-carbon aldehydes. Also, notice that cells 52–59 were activated by the unsaturated and unsaturated-branched 8-carbon aldehydes, but not by octanal. Overall, analysis of the pharmacological profiles of cells tested with this panel of aliphatic aldehydes distinguished at least two different types of aldehyde receptor: those that behave like OR-I7 (i.e. are activated by saturated and unsaturated aldehydes) and those that are more narrowly tuned (i.e. are not activated by the unsaturated analogues, or are activated only by aldehydes with an 8-carbon chain).

Geranial can distinguish among different octanal receptors

We have previously shown that citral reduced responses to octanal in OR-I7 (Araneda *et al.* 2000). Thus, we tested the possibility that citral could exert differential effects in other receptors for aldehydes. Citral is a racemic mixture of two isomers, geranial and neral, which differ in the orientation of the methyl group at carbon-3, raising the possibility that these isomers might contribute differently to the antagonizing effect. First, we tested each isomer (100 μM) against octanal (10 μM) in cells expressing OR-I7. Both isomers almost completely abolished the response to octanal in a reversible fashion (Fig. 4*A*). Although neral had a slightly smaller antagonistic effect compared to geranial (Fig. 4*B*), the difference was not significant

($P < 0.8$), suggesting that at the OR-I7 both isomers are equally effective antagonists. In contrast, in other cells that responded to octanal the responses to geranial varied between 6 and 80% of the response to octanal (average, 25 ± 13%). In these cells coapplication of geranial with octanal resulted in responses that were additive or only slightly reduced (110 ± 25%, Fig. 4*B*, right). In two cells in which geranial did not exhibit a response, the responses to octanal in the presence of geranial were 39 and 100% of the responses to octanal alone (not shown). These results indicate that in different receptors for aldehyde the citral aldehydes either do not bind the receptor, or their binding produces different degrees of antagonism, further delineating differences in the pharmacology of these receptors.

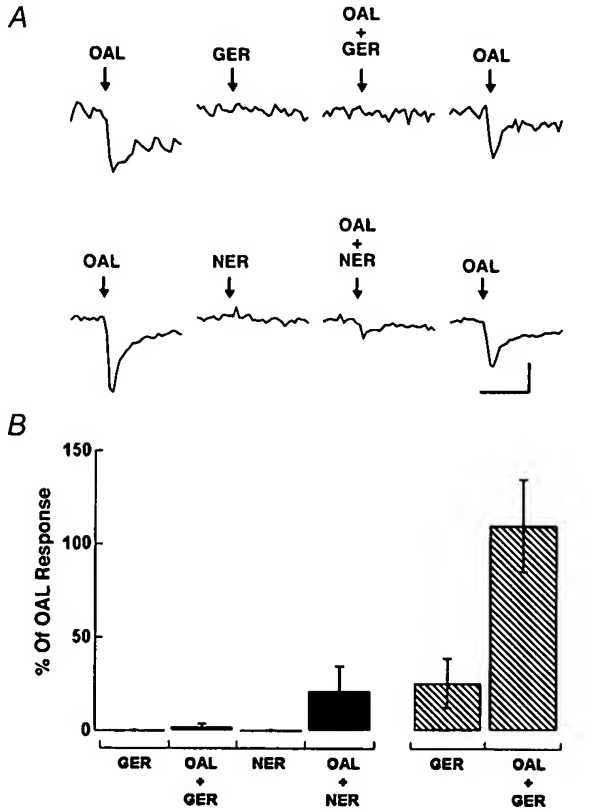

Figure 4. The isomers geranial and neral reduce the responses to octanal at some aldehyde receptors
A, GER (top traces) and NER (bottom traces) reversibly reduced the responses to octanal in cells expressing OR-I7. Both isomers were tested at 100 μM, and in the cells shown here they did not generate a response (see Methods). Calibration bar, 6% $\Delta F/F$ (vertical) and 1 min (horizontal). *B*, not all receptors for aldehydes were blocked by the isomers. Both isomers almost completely reduced the response to octanal in GFP+ cells (filled bars). In contrast, in GFP– cells, GER produced a small response and failed to inhibit the response to octanal (hatched bars; see text).

Different receptors exhibit various sensitivities for aldehydes

Responses to octanal in cells expressing the OR-I7 were dose dependent, exhibiting a threshold for response between 0.1 and 0.3 μM and saturation between 10 and 30 μM. For five cells with complete dose data, a fit of the Hill equation yielded an EC_{50} of 1.9 ± 0.5 μM and a Hill coefficient of 2.8 ± 0.1 (Fig. 2*B*). Compared with the EC_{50} obtained for other receptors using similar recording techniques, this value is relatively low and suggests that OR-I7 may be a high affinity receptor for octanal (Kajiya *et al.* 2001; Bozza *et al.* 2002). In some cells we observed a decrease in responses at high concentrations of octanal, which could be due to desensitization of the receptor at higher concentrations (i.e. 30 μM). Similarly, we found that in other cells responding to octanal its effect was also dose dependent, with thresholds for octanal responses ranging from 0.1 to 30 μM. As these cells correspond to a heterogeneous group of ORs the data could not be averaged, but the response from one cell, which had a threshold for octanal at 10 μM, is plotted in Fig. 2*B* for comparison. These results indicate that multiple receptors bind aldehydes, albeit with different affinities (see below).

In a group of 2301 GFP– isolated OSNs (see Methods) we found 144 (6%) octanal-responding cells at 30 μM (a saturating concentration for OR-I7). Assuming there is a more or less equal representation of ORs in sensory neurones, and that each OSN expresses only 1 out of ~1200 receptors in the rat (S. Firestein, unpublished observations), we might expect that ~70 receptors are activated by octanal at 30 μM (Ma & Shepherd, 2000; Hamana *et al.* 2003). Increasing the concentration of octanal to 1000 μM elicited a response in 47/220 cells (21%), corresponding to ~250 octanal sensitive receptors, representing the recruitment of 180 additional ORs (data not shown). These findings are in general agreement with observations using optical imaging and fMRI, which show that high concentrations of aldehydes activate a large number of glomeruli in the olfactory bulb (Wachowiak & Cohen, 2001; Fried *et al.* 2002; Xu *et al.* 2003).

Molecular range of other aldehyde receptors

Given this apparently large number of receptors that can detect aldehydes it would be of interest to determine if they can be discriminated pharmacologically. To further distinguish the profile of different receptors for aldehydes we challenged a group of octanal-responding neurones to a select panel of eight other odorants. This panel consisted of aliphatic and aromatic aldehydes, as well as non-aldehyde

compounds (see Fig. 1). All of the compounds were tested at 30 μM. Based on our dose–response relation for OR-I7 and other receptors (see above), this concentration should allow us to detect both low and high affinity receptors. While some of these odorants might activate the receptors at lower concentrations, the issue of specificity is better addressed at mid-range concentrations where a non-response indicates significant selectivity of the receptors.

Analysis of the response profiles of 55 cells reveals several receptor types, all of which are activated by octanal, but otherwise possess clearly distinct pharmacological profiles. Examples of odorant responses for some of those cells are shown in Fig. 5. Some receptors, illustrated by cell 1, have a broad spectrum of activity, responding to all the compounds in this panel. In contrast, other receptors were activated only by octanal, suggesting that they are very narrowly tuned as even 2,4-octadienal failed to activate them (cell 47). The remaining cells were distributed along a continuum of profiles, from those activated by at least two molecules to those cells that were activated by several compounds (cells 37 and 8, respectively). In addition to differences in the pattern of activation between OSNs there were also differences in the size of the responses to the various odorants in the same OSN. For example cell 8 exhibited responses to citral or octanol that were smaller than octanal, while its response to cinnamaldehyde was larger (Fig. 5). This suggests differences in affinity and/or efficacy for these compounds at a particular receptor.

To classify the pharmacological profiles from a large number of cells and thus gain further insight into the structure–activity relations among these different receptors we employed cluster analysis, similar to that utilized in analysis of DNA microarrays. Each member of the panel, acting as a molecular probe, is analogous to a gene in an array. Because the components of the panel were compared at a uniform concentration, the profile of each cell was expressed in a binary code as presence or absence of a response (see Methods). As shown in Fig. 6, inspection of the cluster analysis for this set of cells revealed two distinct groups, possessing broadly (cluster A) and narrowly (cluster D) tuned molecular ranges. Furthermore, 10 of the response patterns were observed in more than one cell, suggesting that these cells express the same or a very closely related receptor. Altogether we observed 33 profiles and, based only on patterns that appeared more than once, we can distinguish at least 10 distinct octanal receptor types using only this panel of

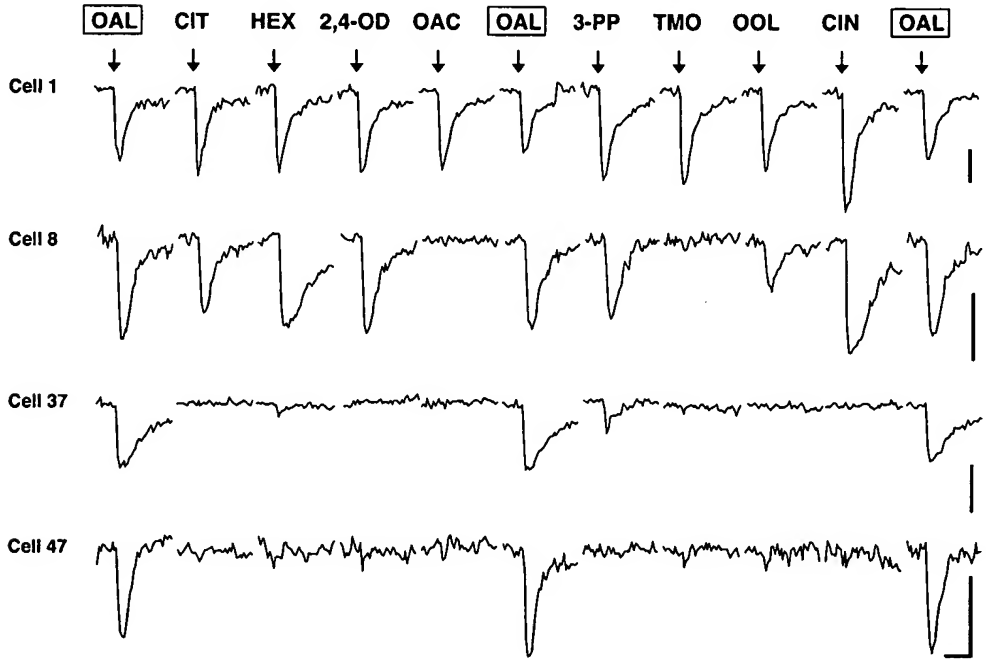

Figure 5. Cells exhibit distinct response profiles to a test panel of odorants
Odorant responses of a selected group of cells stimulated with a panel of 9 odorants. Responses to the different odorants were compared to a test application of OAL. Cells exhibited various profiles of activity, from narrowly tuned (cell 47) to broadly tuned (cell 1). In a few cells some of the components in the panel produced larger responses than octanal (i.e. CIN in cells 1 and 8). All odorants were tested at 30 μM. For all cells the calibration bar is 6% $\Delta F/F$ (vertical) and 1 min (horizontal).

eight odorants. Like the group of cells shown in the lower half of Fig. 3, 67 cells that did not respond to octanal but responded to other components of the panel exhibited particularly narrow tuning (not shown). Overall, of 122 cells that responded to at least one component of the panel, 3% of the cells responded to all the compounds while 39% of the cells responded to only one compound.

Cell 27, activated only by octanal and its unsaturated analogue 2,4-octadienal, exhibited the expected profile of OR-I7 based on our previous work. However, unlike OR-I7, nearly half of the octanal-responding cells (28/55) discriminated between octanal and 2,4-octadienal. Those receptors were more narrowly tuned, with 27/28 cells responding to three or fewer additional components of the panel. Once again many cells responded to citral (22/55), and most of them had a broad tuning profile, with the exception of cell 38, which (besides octanal) was activated only by citral and 3-phenyl propanal. Cell 20 exhibited a similar profile to that described by Bozza *et al.* (2002) for an OR-I7 expressed in transgenic mice; it was activated by octanal, citral, cinnamaldehyde and 2,4-octadienal (though these authors did not test the latter). Also, cluster B was activated by octanal and cinnamaldehyde, but not by 2,4-octadienal or citral. We suggest that these receptors may be closely related to OR-I7.

The alcohol (27/55) and the acid (10/55), which did not activate OR-I7, did activate several octanal-sensitive receptors and in general these receptors had broader tuning

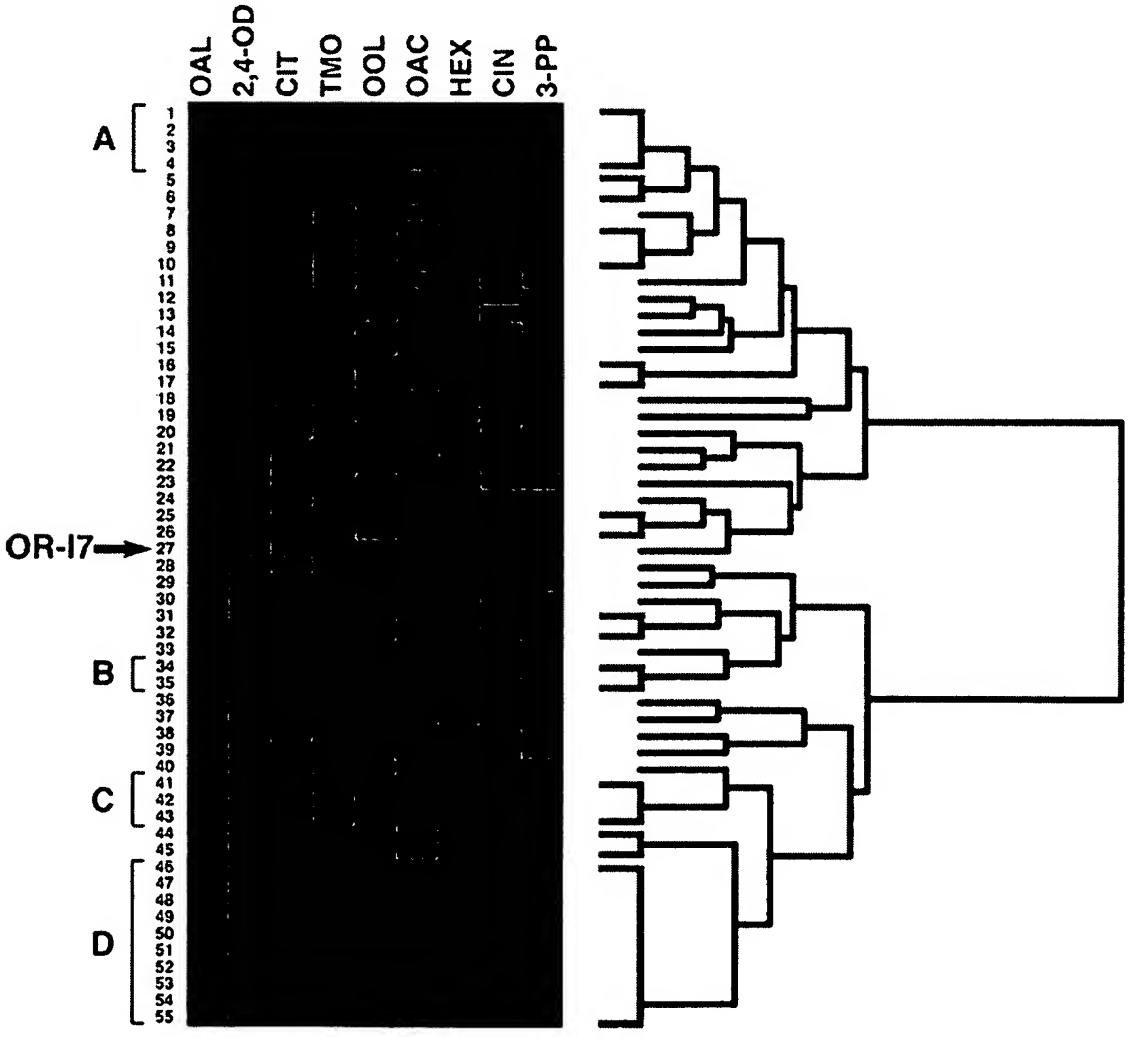

Figure 6. Cluster analysis reveals several aldehyde receptor types
Cluster analysis of 55 octanal responding cells. Cell responses were transformed into a binary profile, which was then used for the cluster analysis (see Methods). Responses are indicated in red and no response in black. There were 33 distinct response profiles, and 10 of these profiles appeared in more than one cell. A few cells responded to all the odorants (cluster A), and a large number only to OAL (cluster D). Cluster C was stimulated only by octanal and TMO, while cell 27 had the expected profile of OR-I7. All odorants were tested at 30 μM.

profiles. Interestingly, we found that the majority of the receptors activated by octanoic acid were also activated by octanol (8/10) but the opposite was not generally true. Half of the cells activated by cinnamaldehyde did not recognize the saturated bond at carbon 2 in 3-phenyl propanal. This suggests that other aldehyde receptors, like OR-I7, are sensitive to modifications near the aldehyde group, although differences in the polarization of the aromatic ring in these two molecules could also play a role. It is interesting to note that three cells responded only to octanal and 2,5,7-trimethyl-*trans*-2-octenal, a compound that does not bind to OR-I7 (Fig. 6, cluster C), indicating that in those receptors the methyl group at carbon 2 did not interfere with activity although the methyl at C3 in citral was not tolerated.

Selectivity is maintained at higher concentrations

We reasoned that a 10-fold increase in concentration would allow us to detect the majority of aldehyde-sensitive receptors in our sample and would reveal broadly tuned receptors by their response to most or all of the sample components. Indeed, out of 479 cells tested at 300 μM, 94 (20%) responded to this higher concentration of octanal. However, even at this 10-fold higher concentration, the profiles exhibited a distribution ranging from narrow to wide tuning: at 300 μM 1% of the cells responded to all the components of the panel, while 24% of the cells responded to only one component, suggesting the occurrence of a population of highly selective receptors (data not shown).

In Fig. 7 we show the combined profiles of cells tested at 30 μM (in red) or at 300 μM (yellow), for selected components of the panel. Analysis of the response profiles to the 8-carbon aldehydes present in the panel reveals that all clusters but one (*C*) contained responsive cells from both concentrations (Fig. 7*A*). These clusters included cells that had narrow, intermediate and broad tuning (*D*, *B* and *A*, respectively). Thus, at both concentrations there remained cells that could discriminate among these related aldehydes.

The response profiles of OSNs to the 8-carbon molecules that differ only in the functional group (octanol, octanal and octanoic acid), revealed an additional level of unexpected selectivity. We observed cells stimulated by octanoic acid, octanal and octanol (broadly tuned cells), or by a combination of the alcohol and aldehyde, or the aldehyde and the acid (Fig. 7*B*). However, cells stimulated by only octanol and octanoic acid were never observed. These findings suggest that the interaction of these molecules with the receptor is affected by electronegativity at carbon-1 since the three compounds form a series of increasing electonegativity from octanol to octanoic acid.

Discussion

The problem of detecting and discriminating a large and unpredictable universe of chemical compounds is solved in the olfactory system by using receptors belonging to a family that are better known for their high level of specificity for a small number of hormones and neurotransmitters. The collection of G-protein-coupled receptors (GPCRs) expressed in the olfactory epithelium is numerically large and presumably structurally diverse, but nonetheless seems likely to participate in a combinatorial signalling strategy to produce odour percepts. This notion was in vogue even before the cloning of odorant receptors, but has since gained experimental support from observations in both the olfactory epithelium and the olfactory bulb. In the epithelium Malnic *et al.* (1999) showed that molecules differing only in their functional group activated overlapping but not identical sets of receptors. In the olfactory bulb recent recordings with optical imaging techniques (Rubin & Katz, 1999; Uchida *et al.* 2000; Wachowiak & Cohen, 2001; Fried *et al.* 2002) have confirmed and extended earlier work with 2-deoxyglucose (Leon & Johnson, 2003) and mitral cell recordings (Mori *et al.* 1999) to demonstrate that related odours cause the activation of large and partially overlapping receptor populations.

We have presented data here that largely support these observations and then ask what are the actual numbers of receptors that may be involved in detecting a particular odorant? We have taken a pharmacological approach, using a panel of compounds that differ in specific chemical features, to define receptors by their binding profile, rather than by their nucleotide sequence or synaptic target. In doing so we have been able to approach the more general questions of how many receptors may recognize a particular compound, and how many compounds are recognized by a particular receptor.

To facilitate this analysis we first extended our characterization of a single receptor, OR-I7, using a panel of odorants that had a range of activities, extending from none, to partial, to full. An advantage of the calcium imaging recordings is that, unlike the EOG, we were able to compare different odorants at the same concentration. Nevertheless, we found that compounds that were inactive in the EOG recordings were also inactive in the Ca^{2+} imaging experiments and vice versa, further supporting the reliability of the imaging recording for determining the receptive field of ORs. One characteristic of OR-I7

was the ability of aldehydes unsaturated at carbon 2 to activate this receptor. We found that among the unsaturated aldehydes 2,4-octadienal had a larger response than octanal in the majority of the cells and that 2-octenal was very effective as well, suggesting that a double bond at this position increases affinity or efficacy (or both). Interestingly, the addition of a double bond to a fragrance compound often results in an increased intensity. Assuming that the efficacies for the different aldehydes are similar, we suggest the following affinity ranking for OR I7: 2,4-octadienal > octanal > *trans*-2-octenal > C7.

Only a few studies have analysed the dose–response relation for a single odorant at a mammalian OR; in general the values of EC_{50} obtained are > 10 μM (Kajiya *et al.* 2001; Bozza *et al.* 2002). We find that for octanal this relationship was typically steep, with a threshold concentration for responses at ~0.1–0.3 μM and maximal activity within a narrow concentration range (1 log unit). This type of

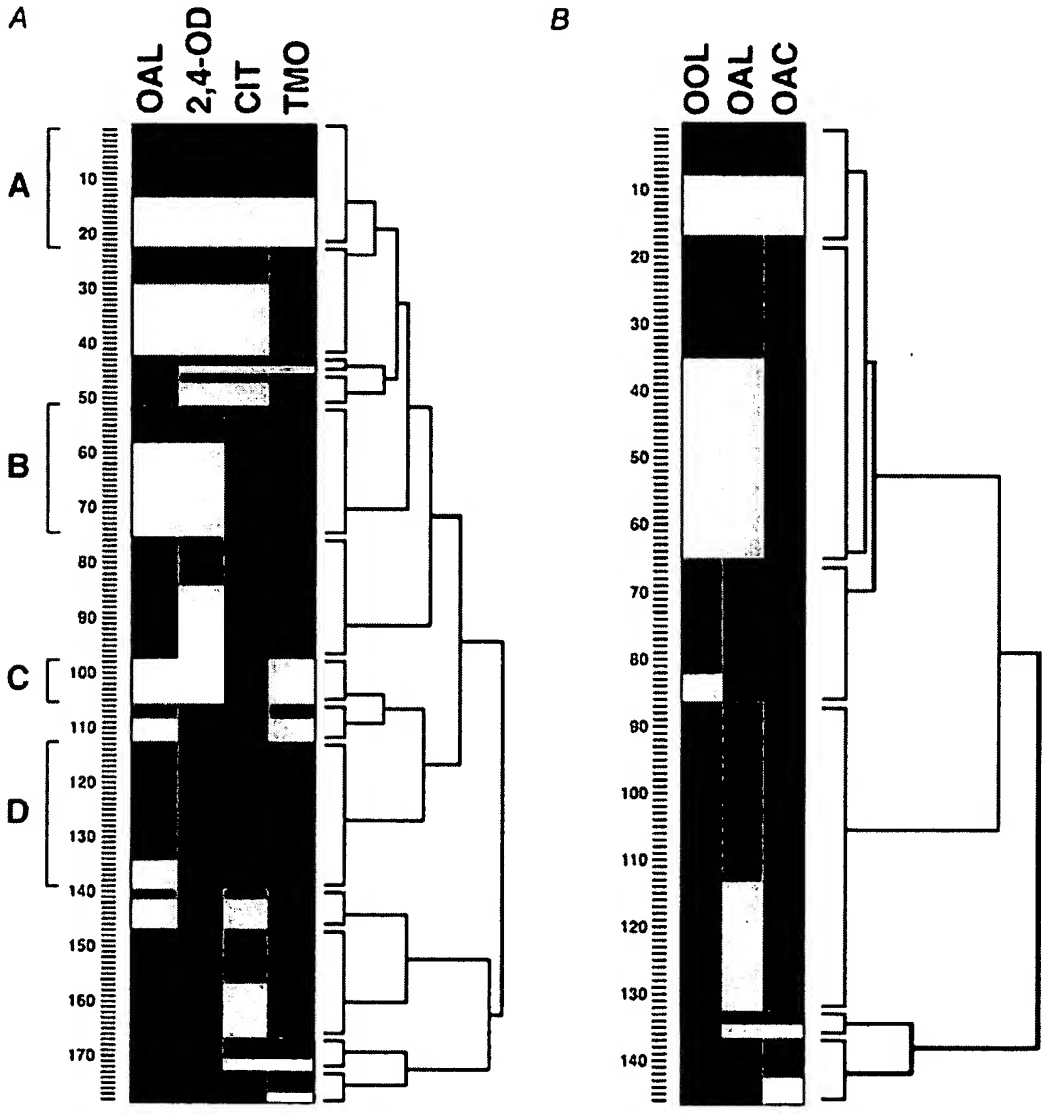

Figure 7. Selectivity of aldehyde receptors is maintained at different concentrations
Cluster analysis for cells tested, at 30 μM (red) or 300 μM (yellow), with a selected group of compounds from the panel. The number of cells included in the analysis is indicated by the labelled grid at the left of each panel, 178 cells in *A* and 146 cells in *B*. *A*, cluster analysis of the response profiles to 8-carbon compounds with different degrees of unsaturation and branching. All of the clusters, except one, included cells from both groups (30 or 300 μM). *B*, cluster analysis of the response profiles to 8-carbon compounds with different functional groups. Cells that responded only to OOL and OAC were not observed at either concentration.

steep relationship has been found in other systems as well, and it may be related to the efficacy of the coupling between the receptor and the transduction pathway in OSNs (Firestein *et al.* 1993). The value of the EC_{50} ($\sim 2\ \mu M$) is the lowest published for a mammalian OR, using Ca^{2+} imaging techniques (Kajiya *et al.* 2001; Bozza *et al.* 2002; but see Levasseur *et al.* 2003), suggesting that this receptor has a high affinity for aldehydes. However, this value may not reflect the true affinity of octanal for the receptor as the observed responses in Ca^{2+} imaging depend on the activation of second messenger pathways, and their stoichiometric relation to the Ca^{2+} signal is unknown. Furthermore, the thresholds for detection of some compounds in mammals are very low (~3.5 p.p.b. for octanal in humans (Cometto-Muniz *et al.* 1998), suggesting that the higher concentrations necessary to activate the receptors in the imaging experiments may reflect technical limitations rather than true receptor kinetics.

In our experiments, citral failed to activate the I7 receptor, but did block the response to octanal, indicating that analogues of octanal can bind to the receptor while possessing lower, or no efficacy at the receptor. This highlights an important and often overlooked feature of ORs, that of antagonism. While well documented in other GPCRs, antagonism has not been regularly reported for ORs (Araneda *et al.* 2000; Oka *et al.* 2003; Spehr *et al.* 2003). However, since olfactory stimuli are commonly a complex mixture of chemicals, the quality of a given stimulus, i.e. its code, will in fact be a combination of activated, partially activated and antagonized receptors. Indeed, commercial fragrances often contain compounds that have no detectable odour of their own, but are known to add to the quality of the mixture. Antagonism at the level of the receptor cells could also explain suppression or inhibition in mixtures of odorants (Laing *et al.* 1989). From the perspective of a neural code both agonistic and antagonistic effects should be considered, especially for complex mixtures containing odorants with related structures – a potential difficulty for assay systems that rely solely on activity for their signal. Finally, pharmacologically the use of antagonists could be useful in classifying receptors, in analogy with their use in other GPCRs.

In regard to the number of receptors that recognize a particular set of ligands we focused on aldehydes because chemicals in this class tend to produce large responses in EOG recordings, suggesting the activation of a large number of receptors. In addition we used a few compounds that were similar in structure to the aldehydes but with different functional groups. With this panel of nine odours we were able to distinguish a surprisingly large number of pharmacological profiles. Overall two types of receptors were present, those with relatively broader tuning profiles, and those that appeared more narrowly responsive. One finding of interest was that half of the responding cells responded to both aldehydes and alcohols, suggesting that many receptors cannot distinguish between these two functional groups, although both compounds maintain a distinctive odour quality, even at high concentrations. This result could explain the observation, in studies with 2-deoxyglucose labelling, that aldehydes and alcohols of the same carbon chain length activated overlapping glomerular modules (Johnson *et al.* 2002). In addition, activation of receptors shared by structurally related or unrelated odorants could explain the phenomenon of cross-adaptation (Dalton, 2000).

Based on pharmacological profiles that were observed in two or more different cells we could distinguish at least 10 different receptors for octanal, and from cluster analysis we estimated that the number of octanal-sensitive receptors could be no lower than 33. This number is higher than that estimated in mice (10–20) by optical imaging of the bulb (Fried *et al.* 2002). The difference could be due to the slightly higher number of predicted functional receptors in rat (S. Firestein, unpublished observations). Adding a few more odours to the panel would probably enable us to pharmacologically distinguish additional receptor types. It is also important to note that in our analysis responses were classified as binary, either yes or no, and no account was taken of different levels of activation. Adding this dimension would further help to discriminate between different receptors. Thus, in the limit we could have identified 55 octanal responsive odour receptors, assuming that every response pattern we observed was representative of a different receptor. Other functional groups, of course, may not be as widely represented in the receptor repertoire.

Our data appear to be inconsistent with a model in which most, if not all, of the receptors are broadly tuned and thus able to recognize molecules with very different chemical structures (Duchamp-Viret *et al.* 1999; Ma & Shepherd, 2000). Indeed we find that the opposite is more likely the case, with many receptors showing surprising levels of selectivity, even among the most similar molecules in the panel. Thus we find selectivity among 8-carbon aldehydes with different degrees of unsaturation or branching, as well as among saturated 8-carbon molecules with different functional groups. Most strikingly we find an apparent selectivity for electronegativity at these functional groups. Another possibility is that this selectivity reflects the nature of hydrogen bonding in the

binding pocket. For example, all three functional groups are potential proton acceptors, but only the alcohol and acid can act as proton donors, suggesting a model in which the hydrogen-bonded protons are donated by residues within the receptor protein rather than by the ligand.

These observations lead us to propose a model in which receptors are relatively specific for particular chemical features and any given compound is discriminated based on its recognition by a large number of receptors, each with an affinity for a particular feature. While this may be a general principle of coding we also expect that there may be compounds recognized with very high affinity by only a few highly specific receptors. These might include burning odours, or those of decomposition, which are known to be recognized at very low concentrations. From an evolutionary perspective, recognition of these sorts of odours by a dedicated labelled-line type of strategy may be more appropriate. Moreover, recognition of the odorant (discrimination threshold) occurs at higher concentrations than the detection threshold (Dalton, 2002), and it may be that high affinity receptors act to signal the presence of an odorant, but determination of the quality requires the combination and recruitment of other receptors.

How can a combinatorial code for odour recognition remain faithful over changes in stimulus concentration? For several compounds odour quality is known to change with concentration. A recent study has shown that in humans the odour quality of three molecules homologous to those included in our panel, heptanal, heptanol and heptanoic acid, changed as their concentration was increased, and none of the odours shared a common quality at high concentrations (Laing *et al.* 2003). However, most odours retain a constant quality over many orders of concentration. We found that octanal activates a large number of receptors and that the number increases appreciably with increasing concentration. This is consistent with data from intrinsic and magnetic resonance imaging studies showing a large number of glomeruli in the bulb activated by aldehydes (Uchida *et al.* 2000; Wachowiak & Cohen, 2001; Fried *et al.* 2002; Xu *et al.* 2003). Increasing concentrations activate additional glomeruli suggesting that additional, presumably lower affinity receptors, are being recruited (Uchida *et al.* 2000; Fried *et al.* 2002). However, this clearly changes the activation pattern and the combinatorial 'code' for that odour. We observe that although more cells respond at higher concentrations, indicating recruitment of new receptors, several cells continue to exhibit narrow profiles, even at high concentrations. This suggests an alternative coding model in which some receptors signify specificity while other receptors may signal concentration. For example there may be receptors with a low affinity and broad sensitivity to many odours. These receptors are only activated by high concentrations, but are not particularly discriminative. Thus the inclusion of these receptors in any pattern of activated receptors simply signals the presence of that odour at a high concentration.

In summary, we have shown evidence that supports the notion that the olfactory system probably uses an array of receptors with different pharmacological tuning, from very specific to quite broad. This places the locus for understanding the code at the olfactory bulb, or higher centres. Whether a glomerular map that reflects an odour code exists in the bulb (Leon & Johnson, 2003), and how the intrinsic circuits within the olfactory bulb modify the signal (Mori *et al.* 1999), are now questions that can be profitably investigated using a relatively compact stimulus set.

References

Araneda RC, Kini AD & Firestein S (2000). The molecular receptive range of an odorant receptor. *Nat Neurosci* **3**, 1248–1255.

Bozza T, Feinstein P, Zheng C & Mombaerts P (2002). Odorant receptor expression defines functional units in the mouse olfactory system. *J Neurosci* **22**, 3033–3043.

Bozza TC & Kauer JS (1998). Odorant response properties of convergent olfactory receptor neurons. *J Neurosci* **18**, 4560–4569.

Buck LB (1992). The olfactory multigene family. *Curr Opin Neurobiol* **2**, 282–288.

Cometto-Muniz JE, Cain WS & Abraham MH (1998). Nasal pungency and odor of homologous aldehydes and carboxylic acids. *Exp Brain Res* **118**, 180–188.

Dalton P (2000). Psychophysical and behavioral characteristics of olfactory adaptation. *Chem Senses* **25**, 487–492.

Dalton P (2002). Olfaction. In *Steven's Handbook of Experimental Psychology: Perception and Motivation*, 3rd edn, pp. 691–746. John Wiley & Sons, New York.

Duchamp-Viret P, Chaput MA & Duchamp A (1999). Odor response properties of rat olfactory receptor neurons. *Science* **284**, 2171–2174.

Firestein S, Picco C & Menini A (1993). The relation between stimulus and response in olfactory receptor cells of the tiger salamander. *J Physiol* **468**, 1–10.

Fried HU, Fuss SH & Korsching SI (2002). Selective imaging of presynaptic activity in the mouse olfactory bulb shows concentration and structure dependence of odor responses in identified glomeruli. *Proc Natl Acad Sci U S A* **99**, 3222–3227.

Gaillard I, Rouquier S, Pin JP, Mollard P, Richard S, Barnabe C *et al.* (2002). A single olfactory receptor specifically binds a set of odorant molecules. *Eur J Neurosci* **15**, 409–418.

Hamana H, Hirono J, Kizumi M & Sato T (2003). Sensitivity-dependent hierarchical receptor codes for odors. *Chem Senses* **28**, 87–104.

Johnson BA, Ho SL, Xu Z, Yihan JS, Yip S, Hingco EE *et al.* (2002). Functional mapping of the rat olfactory bulb using diverse odorants reveals modular responses to functional groups and hydrocarbon structural features. *J Comp Neurol* **449**, 180–194.

Kajiya K, Inaki K, Tanaka M, Haga T, Kataoka H & Touhara K (2001). Molecular bases of odor discrimination: Reconstitution of olfactory receptors that recognize overlapping sets of odorants. *J Neurosci* **21**, 6018–6025.

Kaluza JF & Breer H (2000). Responsiveness of olfactory neurons to distinct aliphatic aldehydes. *J Exp Biol* **203**, 927–933.

Krautwurst D, Yau KW & Reed RR (1998). Identification of ligands for olfactory receptors by functional expression of a receptor library. *Cell* **95**, 917–926.

Laing DG, Legha PK, Jinks AL & Hutchinson I (2003). Relationship between molecular structure, concentration and odor qualities of oxygenated aliphatic molecules. *Chem Senses* **28**, 57–69.

Laing DG, Panhuber H & Slotnick BM (1989). Odor masking in the rat. *Physiol Behav* **45**, 689–694.

Leon M & Johnson BA (2003). Olfactory coding in the mammalian olfactory bulb. *Brain Res Brain Res Rev* **42**, 23–32.

Levasseur G, Persuy MA, Grebert D, Remy JJ, Salesse R & Pajot-Augy E (2003). Ligand-specific dose–response of heterologously expressed olfactory receptors. *Eur J Biochem* **270**, 2905–2912.

Ma M & Shepherd GM (2000). Functional mosaic organization of mouse olfactory receptor neurons. *Proc Natl Acad Sci U S A* **97**, 12869–12874.

Malnic B, Hirono J, Sato T & Buck LB (1999). Combinatorial receptor codes for odors. *Cell* **96**, 713–723.

Mombaerts P (1999). Seven-transmembrane proteins as odorant and chemosensory receptors. *Science* **286**, 707–711.

Mori K, Nagao H & Yoshihara Y (1999). The olfactory bulb: coding and processing of odor molecule information. *Science* **286**, 711–715.

Murrell JR & Hunter DD (1999). An olfactory sensory neuron line, odora, properly targets olfactory proteins and responds to odorants. *J Neurosci* **19**, 8260–8270.

Oka Y, Omura M, Kataoka H & Touhara K (2004). Olfactory receptor antagonism between odorants. *EMBO J* **23**, 120–126.

Rawson NE, Eberwine J, Dotson R, Jackson J, Ulrich P & Restrepo D (2000). Expression of mRNAs encoding for two different olfactory receptors in a subset of olfactory receptor neurons. *J Neurochem* **75**, 185–195.

Rubin BD & Katz LC (1999). Optical imaging of odorant representations in the mammalian olfactory bulb. *Neuron* **23**, 499–511.

Sato T, Hirono J, Tonoike M & Takebayashi M (1994). Tuning specificities to aliphatic odorants in mouse olfactory receptor neurons and their local distribution. *J Neurophysiol* **72**, 2980–2989.

Serizawa S, Miyamichi K, Nakatani H, Suzuki M, Saito M, Yoshihara Y *et al.* (2003). Negative feedback regulation ensures the one receptor-one olfactory neuron rule in mouse. *Science* **302**, 2088–2094.

Sicard G & Holley A (1984). Receptor cell responses to odorants: Similarities and differences among odorants. *Brain Res* **292**, 283–296.

Spehr M, Gisselmann G, Poplawski A, Riffell JA, Wetzel CH, Zimmer RK *et al.* (2003). Identification of a testicular odorant receptor mediating human sperm chemotaxis. *Science* **299**, 2054–2058.

Touhara K (2001). Functional cloning and reconstitution of vertebrate odorant receptors. *Life Sci* **68**, 2199–2206.

Uchida N, Takahashi YK, Tanifuji M & Mori K (2000). Odor maps in the mammalian olfactory bulb: domain organization and odorant structural features. *Nat Neurosci* **3**, 1035–1043.

Wachowiak M & Cohen LB (2001). Representation of odorants by receptor neuron input to the mouse olfactory bulb. *Neuron* **32**, 723–735.

Wetzel CH, Oles M, Wellerdieck C, Kuczkowiak M, Gisselmann G & Hatt H (1999). Specificity and sensitivity of a human olfactory receptor functionally expressed in human embryonic kidney 293 cells and Xenopus Laevis oocytes. *J Neurosci* **19**, 7426–7433.

Xu F, Liu N, Kida I, Rothman DL, Hyder F & Shepherd GM (2003). Odor maps of aldehydes and esters revealed by fMRI in the glomerular layer of the olfactory bulb. *Proc Natl Acad Sci U S A* **100**, 11029–11034.

Yuste R, Lanni F & Konnerth A (2000). *Imaging Neurons: A Laboratory Manual.* Cold Spring Harbor Laboratory Press, New York.

Zhang X & Firestein S (2002). The olfactory receptor gene superfamily of the mouse. *Nat Neurosci* **5**, 124–133.

Zhao HLI, Otaki JM, Hashimoto M, Mikoshiba K & Firestein S (1998). Functional expression of a mammalian odorant receptor. *Science* **279**, 327–242.

Acknowledgements

We thank the Takasago Company (Kanagawa, Japan) for providing neral and geranial, Givaudan-Roure (Vernier, Switzerland) for 2,5,7-trimethyl-2-octenal, and Dr Christian Margot for helpful comments. This work was supported by the NIDCD.